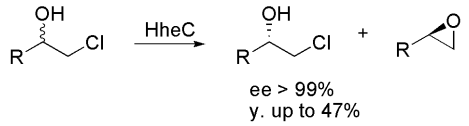
Chapter 3
Enantiopure chloroalcohols via enzymatic kinetic resolution



Alkenyl and heteroaryl chloroalcohols have been obtained in excellent enantiomeric excess (> 99%) by enzymatic kinetic resolution using the haloalcohol dehalogenase HheC. Yields were close to the theoretical maximum for the majority of substrates. Furthermore, the applicability of this methodology on multigram scale has been established. In addition, our efforts to extend the scope of this methodology to other substrates and transformations are described in this chapter.[a]

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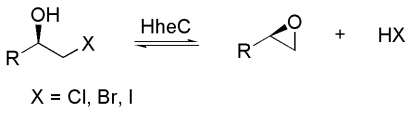
[a] Part of this chapter has been published: R. M. Haak, C. Tarabiono, D. B. Janssen, A. J. Minnaard, J. G. de Vries, and B. L. Feringa, *Org. Biomol. Chem.* **2007**, *5*, 318-323.

3.1 Introduction to haloalcohol dehalogenases

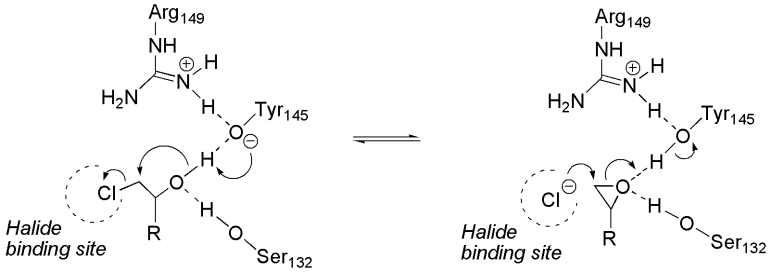
Haloalcohol dehalogenases are enzymes that catalyze the interconversion of haloalcohols and epoxides (Scheme 3.1).[1]



Scheme 3.1 HheC-catalyzed ring closure of haloalcohols.

Haloalcohol dehalogenases can be divided in three groups, called the A, B, and C type.[2] Of these, especially the C type has proven to be a useful biotechnological tool in enantioselective transformations. HheC is a haloalcohol dehalogenase produced by *Agrobacterium radiobacter* AD1, a soil-dwelling bacterium that is able to use halogen-containing organic compounds as its sole carbon source. The enzyme was discovered some years ago, when its role in the degradation pathway of halogenated xenobiotic compounds such as 1,3-dichloro-2-propanol was disclosed.[3]

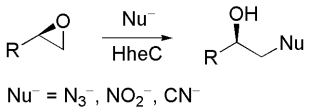
Recently, the structure of HheC has been solved by X-ray crystallography.[4] The active form is a homotetramer, or rather a dimer of dimers, of four identical monomers. Each of the 28 kD monomers has its own active site. However, only the tetrameric state is catalytically active since the monomer is in a catalytically inactive conformation.



Scheme 3.2 Mechanism of HheC-catalyzed ring closure of chloroalcohols, adapted from Refs. 1a and 2.

The kinetics and catalytic mechanism of HheC have been elucidated.[2,4,5] The substrate binds near a catalytic triad (Ser132-Tyr145-Arg149), of which the tyrosine residue activates the hydroxy group of the haloalcohol. Concurrent S_N2-type attack on the vicinal carbon atom by the hydroxy group leads to ring closure and expulsion of the halide anion (Scheme 3.2).

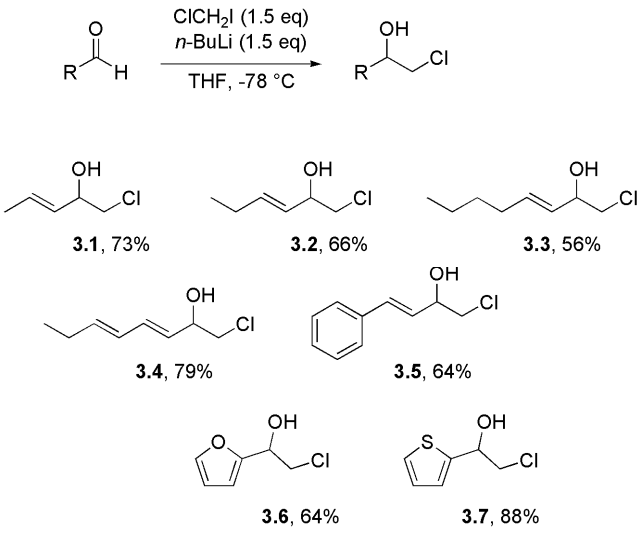
Notably, the biocatalytic potential of HheC has been the subject of investigation[6] and its substrate scope was found to be remarkably wide.[7] HheC catalyzes the reversible ring-closure of various haloalcohols to form epoxides, as well as the irreversible ring-opening of epoxides with a number of non-halide nucleophiles (Scheme 3.3), such as cyanide,[7a] nitrite,[7b] and azide.[7c]



Scheme 3.3 HheC-catalyzed ring opening of epoxides.

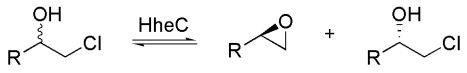
3.2 Diene monoepoxides, chloroalcohols, and their synthesis

Enantiomerically pure epoxides[8] and their immediate precursors, such as chloroalcohols, are valuable synthetic building blocks.[9] Especially functionalized vicinal chloroalcohols like **3.1** - **3.7** (Scheme 3.4) are highly valuable building blocks in synthesis.[10] A number of strategies have been used to prepare these compounds in enantiomerically pure form. Chloroalcohols **3.5** – **3.7** have been prepared by asymmetric transfer hydrogenation of the corresponding chloroketones.[11] There is a recent, detailed study on the preparation of chiral chloroalcohols by ruthenium-catalyzed reduction of aromatic chloroketones.[10] Furthermore, enantiomerically pure **3.6** has been obtained by lipase-catalyzed kinetic resolution of the racemic chloroalcohol,[12] and a route to enantiomerically pure **3.5** by Red-Al reduction of the enantiomerically pure alkyne is known.[13] Though not reported for compounds **3.1** – **3.7**, reduction using alcohol dehydrogenases is a potential method of obtaining these chloroalcohols.[14]



Scheme 3.4 Preparation of functionalized chloroalcohols **3.1** – **3.7**.

Enantiomerically pure **3.1** – **3.4** have not been reported before and a general, convenient, and highly enantioselective method for preparing enantiopure **3.1** – **3.7** is lacking. Kinetic resolution using a haloalcohol dehydrogenase (Scheme 3.5) is an attractive method to obtain these compounds from racemic chloroalcohols.



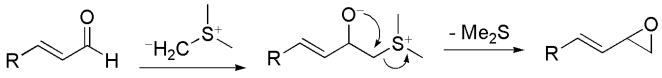
Scheme 3.5 Enzymatic kinetic resolution of chloroalcohols using HheC.

We chose to concentrate on chloroalcohols functionalized with unsaturated and heteraromatic moieties, since these are especially versatile synthetic scaffolds. The compounds studied in this project were prepared using a method published by Lautens and coworkers[15] and are summarized in Scheme 3.4.

The products of ring closure of **3.1** – **3.5** are vinyloxiranes. Like all epoxides, vinyloxiranes (vinylepoxides) are valuable intermediates in synthesis. They are even more versatile than normal epoxides, since in principle three positions are available for nucleophilic attack, depending on the conditions that are chosen.[16,17]

In the preparation of terminal vinylepoxides, one encounters the problem that they are not available by oxidation of terminal dienes. Such oxidation would lead to the epoxidation of the more electron-rich internal double bond.[18]

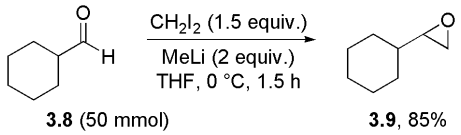
Protocols for the preparation of terminal vinyloxiranes are therefore usually based on the ring closure of an alcohol or alcoholate with a suitable vicinal leaving group. For instance, a common method for their preparation is the addition of dimethylsulfonium methylide to an α,β-unsaturated aldehyde, followed by ring closure to the epoxide and expulsion of dimethylsulfide (Scheme 3.6).[19]



Scheme 3.6 Synthesis of vinyloxiranes from α,β-unsaturated carbonyl compounds.

Unsaturated chloroalcohols can undergo ring closure to yield vinylepoxides.[15] We envisioned that, using a haloalcohol dehalogenase (*vide supra*), this could be done with preference for one of the enantiomers (usually *R*), leading to kinetic resolution.

Interestingly, methodology similar to the one presented in Scheme 3.4 can be used to directly produce epoxides. Thus, if an aldehyde is subjected to diiodomethane in the presence of methyl lithium, epoxides are obtained in good yields, as reported by Concellón *et al.* (Scheme 3.7).[20] Using this method, cyclohexyl oxirane (**3.9**) was prepared in 85% yield (lit. 72%) as a substrate for HheC-catalyzed nucleophilic ring opening with azide in a centrifugal contact separator.[21]

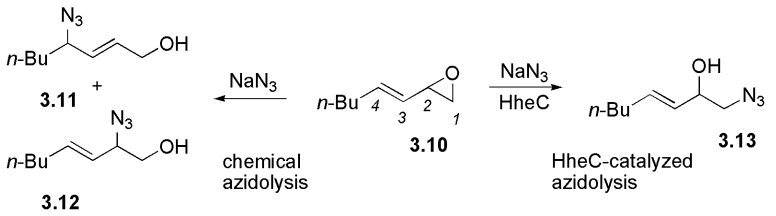


Scheme 3.7 Synthesis of cyclohexyl oxirane.

3.3 Enzymatic nucleophilic ring opening of vinyloxiranes

The initial goal of our investigations was the HheC-catalyzed azidolysis of vinyloxiranes, depicted in Scheme 3.8. Using bulky nucleophiles and under basic conditions, vinyloxiranes are often attacked at the terminal position.[22] However, attack at the 2- or 4-position is frequently observed, especially under conditions that favor formation of the allylic cation.[16] By using halohydrin dehalogenase HheC (from

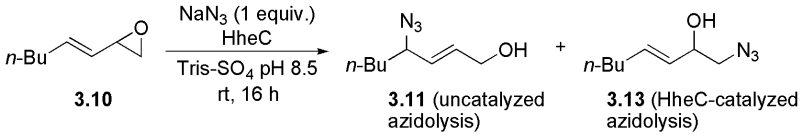
Agrobacterium radiobacter AD1) as catalyst, regioselective azidolysis on the 1-position was expected to be possible. It was anticipated that this would be an enantioselective reaction as well, leading to kinetic resolution. Since 1,2-epoxy-3-octene (**3.10**) is not commercially available, it was synthesized by reaction of hept-2-enal and trimethylsulfonium methylsulfate[23] or trimethylsulfonium iodide.[19]



Scheme 3.8 Reaction pathways in the HheC-catalyzed azidolysis of 1,2-epoxy-3-octene.

In preliminary experiments the enzyme showed some activity, but the blank azidolysis was faster than the enzyme-catalyzed reaction (Table 3.1). It was considered that, by keeping the azide concentration low, it would be possible to improve this ratio, but this turned out not to be the case. Given the disappointing results in terms of reactivity, the enantioselectivity was not further investigated.

Table 3.1 HheC-catalyzed azidolysis of 1,2-epoxy-3-octene (**3.10**).[a]

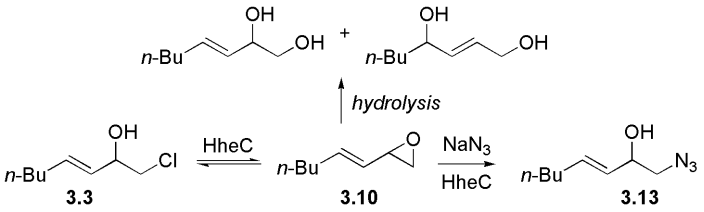


Entry	Addition time azide	Init. rate enzymatic azidolysis (mmol•min^{-1})[b]	Init. rate uncatal. azidolysis (mmol•min^{-1})	Final ratio enzymatic / uncatalyzed
1	at once	1.6 (0.41)	5.9	16/84
2	45 min[c]	1.0 (0.25)	4.0	15/85
3	100 min[c]	0.5 (0.13)	1.6	15/85

a) Conditions: 200 µL of a solution of **3.10** (0.5 M in DMSO) and 500 µl of an aqueous solution of purified HheC (8 mg•ml^{-1}) were dissolved in 20 ml of Tris-SO_4 buffer (200 mM, pH 8.5), after which sodium azide (1 equiv. as a 750 mM aqueous solution) was added. Samples (1.0 mL) were periodically taken from the mixture, extracted with Et_2O, and the resulting samples analyzed by GC; b) In parentheses the initial enzyme activity (in U•mg^{-1}) is given; c) Using a syringe pump.

We investigated if it was possible to start from 1-chloro-3-octene-2-ol (**3.3**) as illustrated in Scheme 3.9. The hydrolysis and blank azidolysis are first order in **3.10**.

The enzyme-catalyzed reaction is governed by Michaelis-Menten kinetics (*vide infra*), so that the rate of reaction is independent of the concentration of **3.10** if the concentration is sufficiently higher than the K_M (see also Equation 3.1). Hence, provided that the K_M is sufficiently low, it could be possible to suppress hydrolysis and uncatalyzed azidolysis of **3.10** while the HheC-catalyzed azidolysis still proceeds at maximum rate.



Scheme 3.9 Enzyme-catalyzed tandem reaction from **3.3** to **3.13**.

It turned out that spontaneous hydrolysis of the epoxide in this case is faster than ring-opening by azide. The observed azidolysis is even more in favour of the uncatalyzed reaction than when the epoxide is directly employed as substrate (enzymatic / uncatalyzed = 96 : 4). However, importantly, in a sample taken after 20 min, only (*S*)-**3.3** could be observed, whereas (*R*)-**3.3** had completely reacted away. Based on this observation, we turned our attention to the enzymatic kinetic resolution of this compound and similar chloroalcohols.

3.4 Enzymatic ring closure of chloroalcohols to epoxides

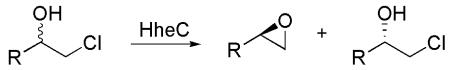
3.4.1 Kinetic resolution of 3.1 – 3.7 on analytical scale

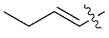
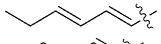
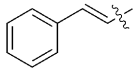
We studied the synthesized chloroalcohols **3.1** – **3.7** as substrates for HheC, initially on analytical scale. The results of this screening are summarized in Table 3.2. Enzymatic activity towards each of the substrates is expressed both as initial enzyme activity ($\mu mol \cdot min^{-1} \cdot mg^{-1}$ of enzyme) and as turnover frequency (s^{-1}). In the last column, the selectivity factor E is given for each of the substrates.

A clear trend in reactivity can be observed with the linear substrates: the shorter the chain, the faster the enzymatic conversion. This is expected on the basis of previous observations.[7] We attribute the observed reactivity pattern to increasing difficulty of the substrate to fit into the active site of the enzyme in a reactive conformation as it gets bulkier. Based on such steric arguments, the reactivity pattern of substrates **3.1** – **3.5** can

be rationalized. Variations in substrate binding between **3.1** – **3.5**, possibly influenced by steric factors or mutual differences in binding affinities, may also partly explain the marked difference in reactivity between the otherwise comparable substrates **3.6** and **3.7**.

Table 3.2 HheC-catalyzed kinetic resolutions of substrates **3.1** – **3.7** on analytical scale.[a]



Entry	Substrate	R =	init. enz. activ.[b] (μmol·min^{-1}·mg^{-1})	TOF (s^{-1})[c]	E[d]
1	**3.1**		48	22.4	>200
2	**3.2**		29	13.5	177
3	**3.3**		8	3.7	>200
4	**3.4**		12	5.6	102
5	**3.5**		10	4.7	>200
6	**3.6**		47	21.9	>200
7	**3.7**		11	5.1	65

a) General conditions: 0.2 mmol scale, 10 mM in Tris-sulfate pH 8.1; b) Initial enzyme activity (μmol of product per min per mg of HheC); c) Per enzyme subunit; d) Obtained by fitting measured data points (concentration *v.* time) against the mathemetical curves for competitive Michaelis-Menten kinetics using MicroMath® Scientist®.

The enantioselectivity of these transformations is high in all cases, and even the lowest E observed (65, Table 3.2, entry 7) is excellent for a kinetic resolution.

Equation 3.1 Competitive Michaelis-Menten kinetics.

a) $$\frac{dR}{dt} = -\frac{V_{\max}^{R} \cdot R}{R + \left(\frac{S}{K_m^S} + 1\right) \cdot K_m^R} - k_c \cdot R$$

b) $$\frac{dS}{dt} = -\frac{V_{\max}^{S} \cdot S}{S + \left(\frac{R}{K_m^R} + 1\right) \cdot K_m^S} - k_c \cdot S$$

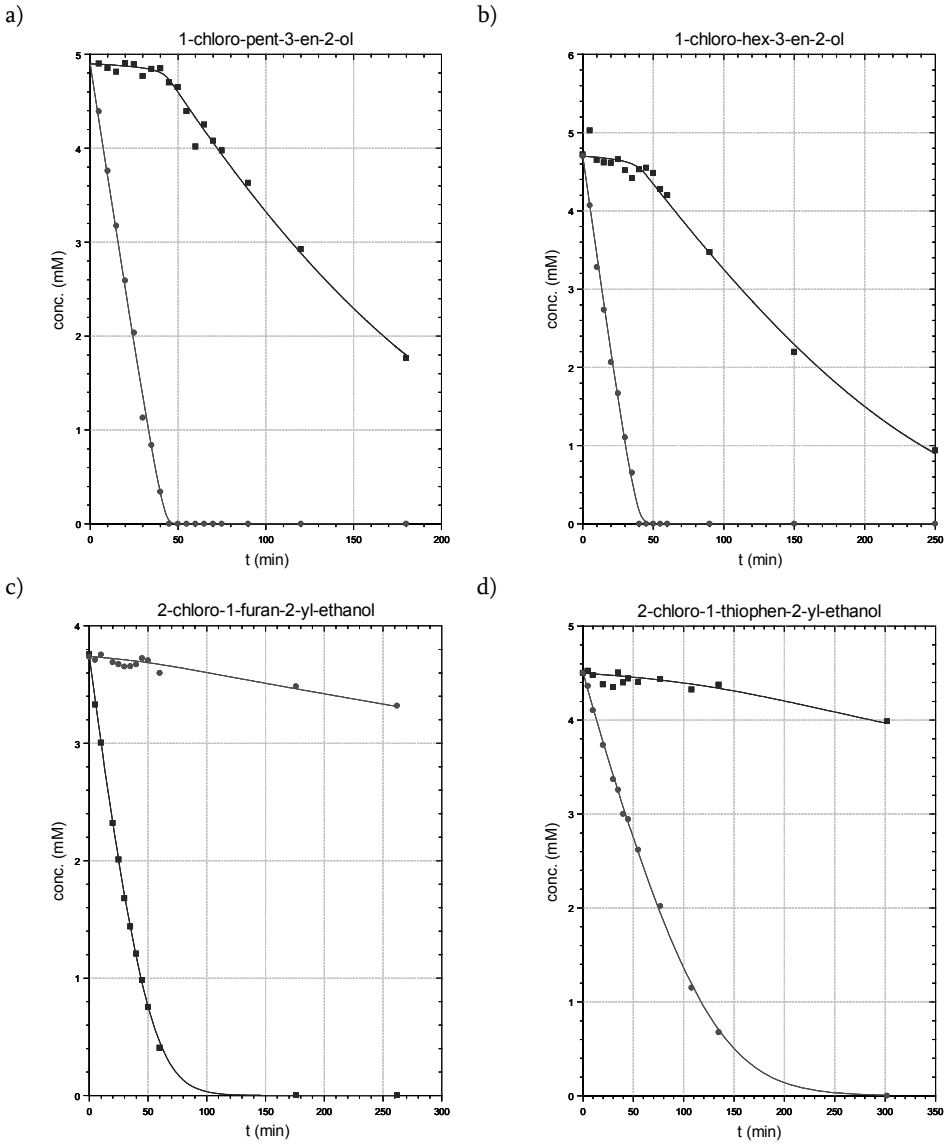


Figure 3.1 Progress curves for enzymatic conversion of **3.1** (a), **3.2** (b), **3.6** (c), and **3.7** (d).

Equation 3.2 Calculating E from kinetic parameters.

$$E = \frac{V_{\max}^{R} / K_{m}^{R}}{V_{\max}^{S} / K_{m}^{S}}$$

In Equation 3.1a and b, *R* and *S* represent the concentrations of both enantiomers, $V_{max}{}^{R}$, $V_{max}{}^{S}$, $K_M{}^{R}$ and $K_M{}^{S}$ are the relevant Michaelis-Menten parameters, and k_c is the first-order rate constant of chemical hydrolysis. After fitting these equations by numerical integration to the obtained data points,[24] the E-value was calculated from Equation 3.2.[25]

Other methods for calculating the E-value of an enzymatic conversion are given in Equation 3.3. They are not extensively used in this chapter, but are relevant for the studies described in other chapters, as well as for the discussion in general. In Equation 3.3a[25], the conversion and the ee of the product are used to calculate E, whereas Equation 3.3b[26] is independent of conversion and is based on the ee's of both substrate and product. Since these methods are time-independent, they do not take into account possible chemical background reactions.

Equation 3.3 Calculation of E from conversion and ee (a) or ee of both substrate and product (b).

a) $$E = \frac{\ln[(1-c)(1-ee_R)]}{\ln[(1-c)(1+ee_R)]}$$

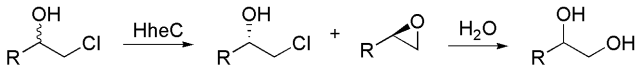
b) $$E = \frac{\ln[(1-ee_S)/(1+ee_S/ee_P)]}{\ln[(1+ee_S)/(1+ee_S/ee_P)]}$$

As is shown in Figure 3.1, the reaction rate of the slow-reacting *S*-enantiomer can be appreciable. This is the case especially for the linear substrates **3.1** – **3.4**. Naturally, this behavior should be taken into account when these reactions are to be performed on preparative scale (*vide infra*).

3.4.2 Kinetic resolution on preparative scale

Having established the remarkable efficiency of this enzymatic kinetic resolution, we set out to transform substrates **3.1** and **3.3** – **3.7** on preparative scale. In view of the excellent selectivities, our initial aim was to isolate both the remaining enantiomerically pure chloroalcohol and the produced epoxide.

Preparative scale reactions were performed on 2.0 mmol scale at a concentration of 10 mM in Tris-sulfate buffer, *i.e.*, analogous to the analytical reactions. In this manner it proved possible to isolate enantiomerically pure chloroalcohols (ee >99%) in fair to high yields (Table 3.3, entries 1 – 4). Our attempts to isolate the produced epoxides as well failed, since it turned out that they rapidly hydrolyse *in situ* to form the corresponding diols (Scheme 3.10).



Scheme 3.10 Hydrolysis of the product epoxides in HheC-catalyzed ring closure of halohydrins.

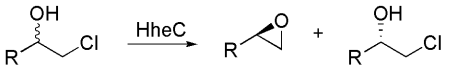
Attempts were made to suppress this hydrolysis by performing the reaction in a two-phase system (Table 3.3, entries 5 and 6), but even in those cases hydrolysis proved inevitable. The role of this spontaneous hydrolysis will be examined in more detail further on.

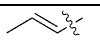
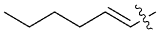
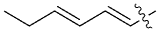
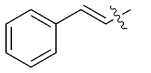
Accordingly, our efforts focused on obtaining the enantiomerically pure halohydrins, which were isolated with excellent enantioselectivities (Table 3.3). In general, the yields were good, allowing for the isolation of **3.1** and **3.5** – **3.7** in 40 – 47% yield. Using substrates **3.3** and **3.4**, yields were around 30%. The cause of these lower yields is not clear.

The absolute configuration of the remaining chloroalcohols was *S* in all cases, in agreement with previous studies showing that HheC is *R*-selective for the majority of substrates.[6,7] A variety of methods were used to elucidate the absolute configuration of the slow-reacting enantiomers of the chloroalcohols. The absolute configuration of (*S*)-(*E*)-1-chloro-4-phenyl-but-3-en-2-ol ((*S*)-**3.5**) could be deduced from a crystal structure using the Bijvoet method with chloride as heavy atom (CCDC 605888).[27] The optical rotations of (*S*)-**3.6** and (*S*)-**3.7** were identical to literature values for these compounds.[11,12] Finally, (*S*)-**3.1**, (*S*)-**3.3**, and (*S*)-**3.4** were hydrogenated to **3.14** and **3.15** using Wilkinson's catalyst and dihydrogen (Scheme 3.11). For these saturated

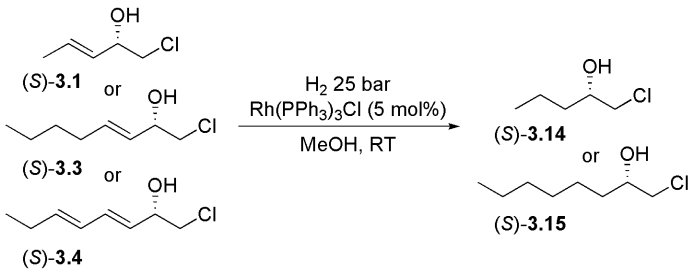
analogues, specific rotations are known, allowing the absolute configuration to be established by structural correlation.[28]

Table 3.3 Enzymatic kinetic resolutions of unsaturated and heteroaromatic vicinal chloroalcohols on preparative scale.



Entry	Substrate	R =	Chloroalcohol (y[a], ee, conf.)	Diol (y[a])	E[d]
1	**3.1**[b]		40%, >99%, *S*	n.i.[c]	>200
3	**3.3**[d]		31%, >99%, *S*	n.i.[c]	177
4	**3.4**[e]		29%, >99%, *S*	24%[f]	>200
5	**3.5**[d]		47%, >99%, *S*	19%[f]	102
6	**3.6**[g]		42%, 98.5%, *S*	n.i.[c]	>200
7	**3.7**[h]		47%[i], >99%, *S*	49%[i]	>200

a) Isolated yield (based on 50% maximum); b) 1.0 mmol scale, 10 mM in Tris-sulfate buffer (pH 8.1); c) Not isolated; d) 2.0 mmol scale, 10 mM in Tris-sulfate buffer (pH 8.1); e) 1.5 mmol scale, 10 mM in Tris-sulfate buffer (pH 8.1); f) Non-optimized yield of a mixture of diols; g) 16 mmol scale, 1 : 1 toluene / Tris-sulfate (pH 8.1); h) 120 mmol scale, 1 : 10 toluene / Tris-sulfate (pH 8.1); i) crude yield.



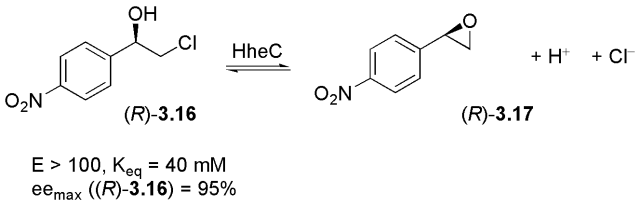
Scheme 3.11 Hydrogenation of (*S*)-**3.1**, (*S*)-**3.3**, and (*S*)-**3.4**. to (*S*)-**3.14** and (*S*)-**3.15**.

We were especially interested in the possibilities of performing this resolution as a preparative procedure. It was indeed possible to perform this kinetic resolution on multigram scale (Table 3.3, entries 5 and 6). For instance, starting from 20.9 g of racemic 2-chloro-1-thiophen-2-yl-ethanol (**3.7**), 9.8 g (94% of the theoretical yield) of

(*S*)-**3.7** was obtained with an excellent ee of >99%. No modifications to the enzyme (*e.g.* immobilization) were needed to achieve these results and the resulting compounds are stable for several months at 4 °C. To avoid the use of excessive amounts of buffer solution, the latter two reactions were performed in a two-phase system of toluene and Tris-buffer (pH 8.1).

3.5 Hydrolysis of epoxides during enzymatic kinetic resolution

It was observed that the epoxides that were produced during HheC-catalyzed kinetic resolution, almost immediately hydrolyzed spontaneously to the corresponding diols (*vide supra*). This was initially perceived as a drawback of the system, since it interfered with our intentions of isolating both the remaining chloroalcohols and the epoxides. However, comparison of our results with those previously reported for the related compound 2-chloro-1-(4-nitro-phenyl)-ethanol (**3.16**) disclosed a more positive role of hydrolysis in the kinetic resolution of chloroalcohols.



$$K_{eq} = \frac{[epoxide]\cdot[HCl]}{[chloroalcohol]}$$

Scheme 3.12 Incomplete conversion of (*R*)-2-chloro-1-(4-nitro-phenyl)-ethanol ((*R*)-**3.16**).

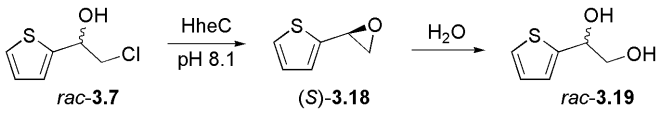
As illustrated in Scheme 3.12, when a kinetic resolution is performed on *rac*-**3.16**, the *R*-selective enzyme HheC converts the *R*-enantiomer at a much faster rate than the *S*-enantiomer. However, the K_{eq} of the equilibrium between (*R*)-**3.16** and (*R*)-**3.17** is 40 mM,[7c] in other words when equilibrium is reached, the ratio between chloroalcohol and epoxide will be about 2.5 / 97.5, indicating that a small percentage of (*R*)-**3.16** will always remain present if the epoxide is in equilibrium with the chloroalcohol. In this case, the maximum ee of the remaining chloroalcohol will be about 95% (in practice: 92%).[6e,7c]

The results obtained in this study indicate that the spontaneous hydrolysis of the formed epoxide results in a shift of the chloroalcohol-epoxide equilibrium to the

product side by removing the epoxide from the reaction, thus ensuring excellent ee's of the remaining chloroalcohols.

3.5.1 The role of hydrolysis in HheC-catalyzed enzymatic kinetic resolution

Contrary to our expectations, in the case of substrate **3.7** (Table 3.3, entry 6) hydrolysis led to the formation of racemic diol (Scheme 3.13), probably resulting from approximately equal rates for nucleophilic attack on the terminal and internal carbon atom of the (enantiomerically pure) epoxide.[29]



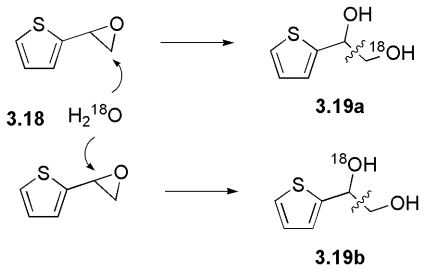
rac-**3.7** (*S*)-**3.18** *rac*-**3.19**

Scheme 3.13 Formation of racemic diol **3.19** from enantiopure epoxide **3.18**.

Under the mildly basic reaction conditions of the enzymatic kinetic resolution, it is unlikely that racemization results through the intermediacy of a (hetero)benzylic carbocation. A better explanation for the formation of racemic diol is a 1 : 1 ratio of attack on the sterically favored terminal carbon atom of the epoxide and the electronically favored benzylic carbon atom.[29] Evidence for this hypothesis could be provided by the following experiments: ^{18}O labeling experiments (Scheme 3.14) and pH dependence on product distribution (Scheme 3.15).

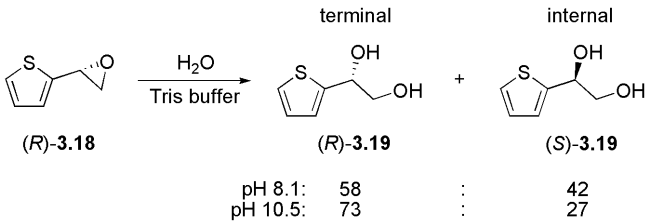
In MS (EI+) measurements, the $[M - CH_2OH]^+$ peak has a high abundance. The mass of this fragment will be 113 for diol **3.19a**, resulting from terminal attack of water, and 115 for diol **3.19b**, resulting from attack of labeled water on the benzylic position. By measuring the ratio of these fragments, one can determine the regioselectivity of the epoxide ring opening.

Thiophen-2-yl-oxirane (**3.18**) was synthesized by reaction of thiophene carboxaldehyde and trimethylsulfonium iodide in the presence of potassium hydroxide[30] and was isolated in 60% yield after Kugelrohr distillation. The hydrolysis experiment was performed by adding 5 μL of epoxide to 30 μL of $H_2{}^{18}O$. These conditions do not completely reflect the experimental conditions of enzymatic kinetic resolution, since in this case the pH is around 7, whereas the enzymatic kinetic resolution is conducted at pH 8. Diols **3.19b** and **3.19a** were obtained in a 4 : 1 ratio. This proves that attack taking place at the terminal position is a significant pathway, even under neutral conditions.



Scheme 3.14 Ring opening of thiophenyl oxirane by $H_2{}^{18}O$.

Experiments on the pH-dependence of the hydrolysis of (*R*)-**3.18** are in agreement with the findings in the previous paragraph. Enantiomerically pure (*R*)-**3.18** was obtained by K_2CO_3-catalyzed ring closure of (*S*)-**3.7** in acetone.[b] Subsequently, hydrolysis experiments were conducted at different pH values (Scheme 3.15). At pH 8.1, nearly racemic **3.19** was obtained, whereas at pH 10.5 an excess of (*R*)-**3.19**, the product with retention of configuration, is obtained. This indicates that the proportion of diol resulting from attack on the terminal carbon atom of the epoxide, increases with increasing pH.[31]



Scheme 3.15 pH-dependence of the hydrolysis of (*R*)-**3.18**.

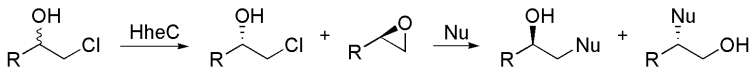
To summarize, nucleophilic attack at the terminal carbon atom of **3.18** is a significant pathway, even at pH values lower than during enzymatic kinetic resolution. Furthermore, the ratio of terminal and internal attack increases with increasing pH. In conclusion, competition between attack on the terminal and internal carbon atom on

[b] Various bases (NaH, NaOH, K_2CO_3), solvents (DMF, THF, Et_2O, IPA, H_2O) and conditions (presence or absence of additives like NaI or silver salts) were examined. Reproducible results were obtained using K_2CO_3 in acetone.

the epoxide is the most likely explanation for the formation of racemic **3.19**. It is possible that other mechanisms are involved in the hydrolysis of the other substrates.[29]

3.5.2 Trapping of epoxide to prevent hydrolysis

Spontaneous hydrolysis of the epoxide product limits the applicability of this – in principal – highly efficient kinetic resolution. To prevent hydrolysis of the formed epoxide, it might be feasible to convert it *in situ* to a product which is more stable towards hydrolysis, using a strong nucleophile (Scheme 3.16). Besides finding a nucleophile that is reactive enough to accomplish this under the given reaction conditions, two selectivity issues have to be addressed: the nucleophile should react selectively with the epoxide as opposed to the chloroalcohol (chemoselectivity) and it should favor attack on the terminal epoxide carbon as opposed to the internal carbon atom (regioselectivity).

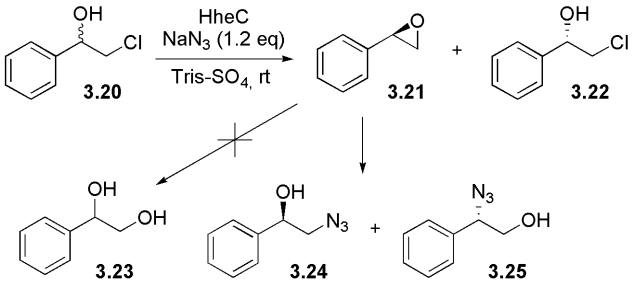


Scheme 3.16 Trapping of epoxide to prevent hydrolysis.

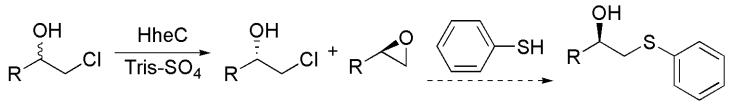
If a nucleophile such as azide, nitrite or cyanide is used, the ring opening reaction will also be enzyme-catalyzed.[32] An advantage of this approach is the regioselectivity of HheC-catalyzed azidolysis of epoxides. Exclusively the terminal adduct will be produced (Scheme 3.17). However, for chloroalcohol **3.20** it was experimentally verified that the enzymatic ring opening of the epoxide with azide is two orders of magnitude slower than the enzymatic ring closure of the chloroalcohol under the same conditions.

The specific enzyme activity is 14.1 μmol·min^{-1}·mg^{-1} for the ring closure of the chloroalcohol (**3.20** → **3.21**) and 0.19 μmol·min^{-1}·mg^{-1} for the ring opening with azide (**3.21** → **3.24**), at their respective pH optima. Therefore, the epoxide accumulates to such an extent that spontaneous hydrolysis becomes inevitable.[33]

In a different approach, thiophenolate was used as the nucleophile (Scheme 3.18). Under the reaction conditions for enzymatic kinetic resolution (pH = 8), thiophenol will be deprotonated (pKa = 6.6) and hence reactive enough to attack the epoxide. Moreover, it is known from the literature that nucleophilic attack of thiophenols on epoxides can be highly regioselective.[34]

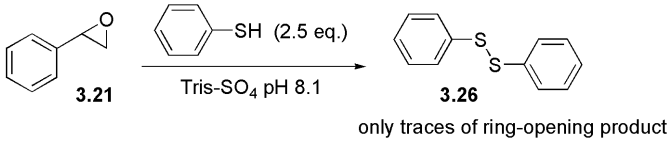


Scheme 3.17 Trapping of epoxide **3.21** to prevent hydrolysis.



Scheme 3.18 Attempted trapping of epoxide by thiophenol.

A difficulty we encountered when testing this approach, was the undesirable oxidative dimerization shown in Scheme 3.19. It was tried to suppress this side-reaction by working under oxygen-free conditions, but dimer **3.26** remained the main product.



Scheme 3.19 Formation of **3.26** during attempted thiophenol addition to **3.21**.

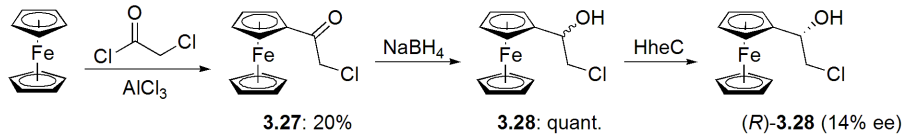
A more thorough screening of sulfur nucleophiles might lead to the desired outcome.

3.6 Limitations of this methodology

To extend the scope of the kinetic resolution procedure developed so far, a number of other substrates were tested. Furthermore, a variation of this approach was tested, employing formate esters instead of haloalcohols.

After the successful results obtained in the kinetic resolution of unsaturated and aromatic chloralcohols (*vide supra*), we became interested in the possibility of using this methodology for the preparation of the ferrocenyl-derived chloroalcohol **3.28**. Such a chiral chloroalcohol could serve as, for instance, a useful starting material in the preparation of enantiomerically pure ferrocenyl-based ligands for asymmetric catalysis.[35] It would also be the first organometallic compound to act as a substrate in this transformation.

Racemic **3.28** was prepared by Friedel-Crafts acylation of ferrocene with chloroacetyl chloride yielding **3.27** in 20% yield (not optimized).[36] Reduction of the resulting chloroketone **3.27** using sodium borohydride[37] led to chloroalcohol **3.28** in quantitative yield (Scheme 3.20).

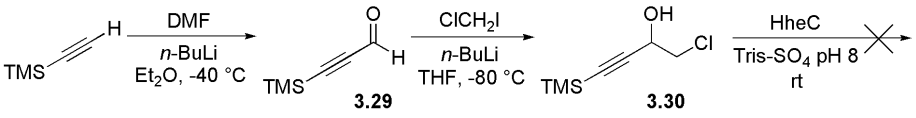


Scheme 3.20 Synthesis and enzymatic kinetic resolution of **3.28**.

In the kinetic resolution, the conversion of compound **3.28** turned out to be very slow. A maximum ee of 14% in the starting material was reached. Probable causes for this include the steric bulk of the compound and its very low solubility in the aqueous reaction medium.

Another substrate that did not show efficient kinetic resolution using HheC, was 1-chloro-4-trimethylsilanyl-but-3-yn-2-ol (**3.30**) depicted in Scheme 3.21.

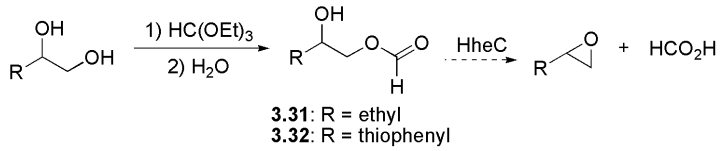
Compound **3.30** was prepared in the usual way from the corresponding aldehyde **3.29**, which in turn was obtained by formylation of trimethylsilyl acetylene using DMF (Scheme 3.5).[38] No reactivity was observed when **3.30** was subjected to the conditions of kinetic resolution that had been effective for substrates **3.1** – **3.7**. As in the case of ferrocenyl-substituted chloroalcohol **3.28**, the lack of reactivity was attributed to steric bulk, in this case due to the presence of the trimethylsilyl moiety. Attempts to remove the TMS-moiety using TBAF were unsuccessful.



Scheme 3.21 Synthesis and attempted enzymatic kinetic resolution of **3.30**.

Another approach that was studied to extend the scope of the developed methodology, included the use of leaving groups other than halides. This idea was prompted by the observation that in the reverse reaction, the ring opening of epoxides, other nucleophiles than halides can be used effectively.

We chose to investigate the ring closure of 2-hydroxyalkyl formates, since formate had been observed to act as a nucleophile, albeit not a very active one,[39] in the reverse reaction. The formates used in this reaction have the advantage that they are easily available from the corresponding diols (Scheme 3.22).[40,41]



Scheme 3.22 Attempted enzymatic ring closure of 2-hydroxyalkyl formates.

However, when tested under the conditions that were successfully applied in the enzymatic kinetic resolution of chloroalcohols, no enzyme activity was observed for the ring closure of formates **3.31** and **3.32** (Scheme 3.22).

3.7 Conclusions

In conclusion, a highly efficient kinetic resolution protocol was developed for functionalized vicinal chloroalcohols. The majority of these compounds have not been reported before in their enantiomerically pure form. Various unsaturated and heteroaromatic chlorohydrins were resolved in high yields and with excellent enantioselectivities. This resolution was shown to be effective on multigram scale, making it highly practical as a preparative method.

Limitations of this methodology include the poor reactivity of bulky substrates and the need to perform this reaction in aqueous solution. The hydrolysis of the epoxide

product that is observed in this reaction, might be instrumental in obtaining high ee's of the chloroalcohols, but limits the applicability of this method.

3.8 Experimental section

3.8.1 General remarks

Starting materials were purchased from Aldrich or Acros and used as received unless stated otherwise. All solvents were reagent grade and, if necessary, dried and distilled prior to use. Demineralized water was used in the preparation of all aqueous solutions.

Column chromatography was performed on silica gel (Aldrich 60, 230-400 mesh). TLC was performed on silica gel 60/Kieselguhr F_{254} or neutral aluminum oxide 60 F_{254} where indicated.

^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR300 (299.97 MHz for ^{1}H, 75.48 MHz for ^{13}C) or a Varian AMX400 (399.93 MHz for ^{1}H, 100.59 MHz for ^{13}C) spectrometer in $CDCl_3$ unless stated otherwise. Chemical shifts are reported in δ values (ppm) relative to the residual solvent peak ($CHCl_3$, ^{1}H = 7.24, ^{13}C = 77.0). Carbon assignments are based on APT ^{13}C experiments. Splitting patterns are indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad).

Mass spectra (HRMS) were obtained on a Jeol JMS-600H. GCMS spectra were recorded on a Hewlett Packard HP6890 equipped with a HP1 column and an HP 5973 Mass Selective Detector.

GC analysis was performed on a Shimadzu GC-17A or a Hewlett Packard HP6890 spectrometer equipped with the columns indicated for each compound separately.

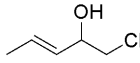
HPLC analysis was performed on a Shimadzu HPLC system equipped with two LC-10AD *vp* solvent delivery systems, a DGU-14A degasser, a SIL-10AD *vp* auto injector, an SPD-M10A *vp* diode array detector, a CTO-10A *vp* column oven, and an SCL-10A *vp* system controller using the columns indicated for each compound separately.

Optical rotations were measured on a Schmidt and Haensch Polartronic MH8 using a 10 cm cell.

3.8.2 Synthesis of substrates 3.1 - 3.7

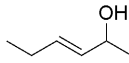
Substrates **3.1** – **3.7** were synthesized according to a literature procedure.[15] Yields, spectroscopic data and chromatographic separation conditions will follow for each of the substrates.

*1-Chloro-pent-3-en-2-ol (**3.1**).*[42]



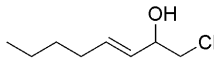
Obtained as a colorless oil (2.21 g; 18.3 mmol; 73%) after flash chromatography (*n*-pentane – Et_2O 4 : 1); ^1H NMR ($CDCl_3$) δ 5.76-5.80 (m, 1H), 5.48 (dd, *J* = 15.4, 6.6 Hz, 1H), 4.26 (m, 1H), 3.58 (dd_{ABX}, *J* = 11.0, 3.7 Hz, $\Delta\nu_{AB}$ = 45.0 Hz, 1H), 3.47 (dd_{ABX}, *J* = 11.0, 7.3 Hz, $\Delta\nu_{AB}$ = 45.0 Hz, 1H), 2.17 (d, *J* = 4.0 Hz, 1H), 1.71 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$) d 129.7 (d), 129.2 (d), 72.3 (d), 49.6 (t), 17.7 (q); MS (EI+) m/z = 122 (M^+), 120 (M^+), 107, 105, 71, 53, 41; chiral GC: Chiraldex B-PM, 30m × 0.25 mm × 0.25 μm, He-flow: 1.1 mL/min, 80 °C isothermic, T_r = 10.2 min (*S*), T_r = 10.9 min (*R*).

*1-Chloro-hex-3-en-2-ol (**3.2**).*



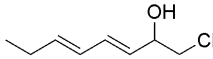
Obtained as a colorless oil (1.77 g; 13.2 mmol; 66%) after flash chromatography (*n*-pentane – Et_2O 6 : 1, gradient to 4 : 1); ^1H NMR ($CDCl_3$) δ 5.84 (dtd, *J* = 15.8, 6.2, 1.1 Hz, 1H), 5.44 (ddt, *J* = 15.4, 6.6, 1.5 Hz, 1H), 4.28 (br, 1H), 3.59 (dd_{ABX}, *J* = 11.0, 3.7 Hz, $\Delta\nu_{AB}$ = 46.1 Hz, 1H), 3.47 (dd_{ABX}, *J* = 11.0, 7.3 Hz, $\Delta\nu_{AB}$ = 46.1 Hz, 1H), 2.22 (d, *J* = 3.7 Hz, 1H), 2.06 (qdd, *J* = 7.3, 6.6, 1.5 Hz, 2H), 0.98 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 136.4 (d), 127.0 (d), 72.3 (d), 49.7 (t), 25.2 (t), 13.1 (q); MS (EI+) m/z = 134 (M^+), 105, 85, 67, 55; HRMS (EI+) calc.: 134.0498, measured: 134.0491; chiral GC: Chiraldex B-TA, 30m × 0.25 mm × 0.25 μm, He-flow: 1.0 mL/min, 85 °C isothermic, T_r = 16.7 min (*S*), T_r = 18.0 min (*R*).

*1-Chloro-oct-3-en-2-ol (**3.3**).*[15,43]



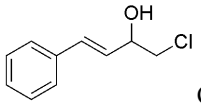
Obtained as a colorless oil (903 mg; 5.55 mmol; 56%) after flash chromatography (*n*-pentane – Et_2O 6 : 1, R_f = 0.32); ^1H NMR ($CDCl_3$) δ 5.79 (dtd, *J* = 15.4, 7.0, 1.1 Hz, 1H), 5.44 (ddt, *J* = 15.4, 6.6, 1.5 Hz, 1H), 4.2 – 4.35 (m, 1H), 3.58 (dd_{ABX}, *J* = 11.0, 3.7 Hz, $\Delta\nu_{AB}$ = 45.0 Hz, 1H), 3.47 (dd, *J* = 11.0, 7.7 Hz, $\Delta\nu_{AB}$ = 45.0 Hz, 1H), 2.19 (d, *J* = 4.2 Hz, 1H), 1.99 – 2.09 (br, 2H), 1.2 – 1.4 (br, 4H), 0.87 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 135.2 (d), 127.9 (d), 72.4 (d), 49.9 (t), 31.9 (t), 31.0 (t), 22.1 (t), 13.9 (q); MS (EI+) m/z = 162 (M^+), 113, 95, 57; chiral GC: CP Chiralsil Dex CB, 25m × 0.25 mm × 0.25 μm, He-flow: 1.0 mL/min, 120 °C isothermic, T_r = 12.6 min (*S*), T_r = 13.0 min (*R*).

*1-Chloro-octa-3,5-dien-2-ol (**3.4**).*



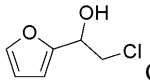
Obtained as a colorless oil (1.29 g; 8.0 mmol; 79%) after flash chromatography (*n*-pentane – Et_2O 7 : 1, R_f = 0.26); ^1H NMR ($CDCl_3$) δ 6.30 (dd, *J* = 15.0, 10.3 Hz, 1H), 6.02 (dd, *J* = 15.0, 10.3 Hz, 1H), 5.79 (dt, *J* = 15.0, 6.6 Hz, 1H), 5.54 (dd, *J* = 15.4, 5.9 Hz, 1H), 4.35 (br, 1H), 3.60 (dd_{ABX}, *J* = 11.0, 3.7 Hz, $\Delta\nu_{AB}$ = 47.5 Hz, 1H), 3.48 (dd_{ABX}, *J* = 11.0, 7.3 Hz, $\Delta\nu_{AB}$ = 47.5 Hz, 1H), 2.23 (d, *J* = 3.7 Hz, 1H), 2.09 (dt, *J* = 13.9, 7.3 Hz, 2H), 0.99 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 138.4 (d), 133.4 (d), 128.1 (d), 128.0 (d), 72.1 (d), 49.6 (t), 25.6 (t), 13.2 (q); MS (EI+) m/z = 162 (M^+), 160 (M^+), 111, 93, 55; HRMS (EI+) calc. 160.0655, measured: 160.0662; chiral GC: CP Chiralsil Dex CB, 25m × 0.25 mm × 0.25 μm, He-flow: 1.0 mL/min, 125 °C isothermic, T_r = 14.8 min (*S*), T_r = 15.6 min (*R*).

*(E)-1-Chloro-4-phenyl-but-3-en-2-ol (**3.5**).*[11,13,15,43,44]



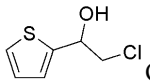
Obtained after flash chromatography (*n*-pentane – Et_2O 5 : 1, R_f = 0.24) as a colorless oil (2.34 g; 12.8 mmol; 64%), which crystallized upon standing; ^1H NMR ($CDCl_3$) δ 7.20 – 7.40 (m, 5H), 6.71 (dd, *J* = 16.1, 1.1 Hz, 1H), 6.19 (dd, *J* = 16.1, 6.0 Hz, 1H), 4.52 (br m, 1H), 3.71 (dd_{ABX}, *J* = 11.0, 3.7 Hz, $\Delta\nu_{AB}$ = 48.9 Hz, 1H), 3.58 (dd_{ABX}, *J* = 11.0, 7.3 Hz, $\Delta\nu_{AB}$ = 48.9 Hz, 1H), 2.38 (d, J = 4.4 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 136.0 (s), 132.7 (d), 128.6 (d), 128.1 (d), 127.2 (d), 126.6 (d), 72.3 (d), 49.6 (t); MS (EI+) m/z = 184 (M^+), 182 (M^+), 133, 115, 105; HRMS (EI+) calc. 182.0498, measured: 182.0507; chiral HPLC: Chiralcel OD, 40 °C, *n*-heptane / IPA 92 : 8, 1.0 mL/min, T_r = 11.5 min (*S*), T_r = 16.0 min (*R*).

*2-Chloro-1-fur-2-yl-ethanol (**3.6**).*[11,12]



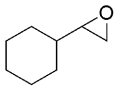
Obtained as a light yellow oil (1.87 g; 12.7 mmol; 64%) after flash chromatography (*n*-pentane – Et_2O 4 : 1, gradient to 3 : 1, $R_{f,3:1}$ = 0.40); for the resolution on 2.3 g scale, different preparations were combined; ^1H NMR ($CDCl_3$) δ 7.39 (s, 1H), 6.36 (s, 2H), 4.93 (m, 1H), 3.83 (m, 2H), 2.53 (d, *J* = 5.5 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 152.6 (s), 142.6 (d), 110.4 (d), 107.6 (d), 68.0 (d), 47.7 (t); MS (EI+) m/z = 148 (M^+), 146 (M^+), 97; chiral GC: Chiraldex G-TA, 30 m × 0.25 mm × 0.25 μm, He-flow: 0.5 mL/min, 120 °C isothermic, T_r = 5.1 min (*R*), T_r = 5.4 min (*S*).

*2-Chloro-1-thiophen-2-yl-ethanol (**3.7**).*[12,45]



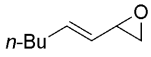
Obtained as a colorless oil (2.86 g; 17.6 mmol; 88%) after flash chromatography (*n*-pentane – Et_2O 4 : 1, R_f = 0.33); for the 20 g scale resolution, this compound was prepared analogously (64%); ^{1}H NMR ($CDCl_3$) δ 7.29 (dd, *J* = 5.1, 1.1 Hz, 1H), 7.03 (ddd, *J* = 3.7, 1.1, 0.7 Hz, 1H), 6.99 (dd, *J* = 5.1, 3.7 Hz, 1H), 5.15 (ddd, *J* = 8.1, 4.0, 0.7 Hz, 1H), 3.80 (dd$_{ABX}$, *J* = 11.4, 4.0 Hz, $\Delta\nu_{AB}$ = 27.4 Hz, 1H), 3.72 (dd$_{ABX}$, *J* = 11.4, 8.1, $\Delta\nu_{AB}$ = 27.4 Hz, 1H), 2.81 (br, 1H); ^{13}C NMR ($CDCl_3$) δ 143.2 (s), 126.9 (d), 125.4 (d), 124.7 (d), 70.2 (d), 50.4 (t); MS (EI+) m/z = 164 (M^+), 162 (M^+), 113; HRMS (EI+, for $C_6H_{37}ClOS$) calc. 163.9877, measured: 163.9881; chiral GC: Chiraldex B-PM, 30 m × 0.25 mm × 0.25 µm, He-flow: 1.1 mL/min, 135 °C isothermic, T_r = 14.2 min (*S*), T_r = 14.8 min (*R*).

*2-Cyclohexyloxirane (**3.9**)*[7a,20]



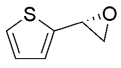
Prepared from cyclohexanecarbaldehyde (**3.8**) as described in Ref. 20. Spectroscopic data were in accordance with the literature.[7a]

*1,2-Epoxy-3-octene (**3.10**)*[15,19]



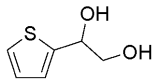
Prepared from *n*-hept-2-enal as described in Ref. 19. Spectroscopic data were in accordance with the literature.[15]

*Thiophenyloxirane (**3.18**)*



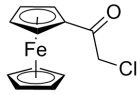
Prepared as described in Ref. 20. (*R*)-**3.18** was prepared by ring closure of (*S*)-**3.7** with K_2CO_3 in acetone overnight at 0 °C → rt. ^{1}H NMR ($CDCl_3$) δ 7.23 (d, *J* = 5.1 Hz, 1H), 7.11 (d, *J* = 3.7 Hz, 1H), 6.96 (dd, *J* = 5.1, 3.7 Hz, 1H), 4.09 (dd, *J* = 4.4, 2.6 Hz, 1H), 3.20 (dd$_{ABX}$, *J* = 5.1, 4.4 Hz, $\Delta\nu_{AB}$ = 58.9 Hz, 1H), 2.98 (dd$_{ABX}$, *J* = 5.1, 2.6 Hz, $\Delta\nu_{AB}$ = 58.9 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 141.3 (s), 127.0 (d), 126.3 (d), 125.2 (d), 51.6 (t), 49.3 (d); chiral HPLC: Chiralcel AS, 40 °C, *n*-heptane / IPA 96 : 4, 1.0 mL/min, T_r = 5.7 min (*S*), T_r = 6.9 min (*R*).

*1-Thiophen-2-yl-ethane-1,2-diol (**3.19**).*[46]



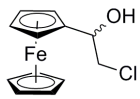
^{1}H NMR ($CDCl_3$) δ 7.26-7.24 (m, 1H), 7.00 – 6.96 (m, 2H), 5.03 (dd, *J* = 7.3, 3.7 Hz, 1H), 3.83 – 3.72 (m, 2H), 3.05 (br, 1H), 2.50 (br, 1H); chiral HPLC: Chiralcel OD, 40 °C, *n*-heptane / IPA 95 : 5, 1.0 mL/min, T_r = 20.4 min (*R*), T_r = 23.3 min (*S*).

*Ferrocenyl chloromethyl ketone (**3.27**).*[36]



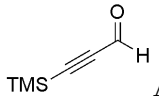
To a flamedried flask under an atmosphere of nitrogen were subsequently added: $AlCl_3$ (400 mg, 3.0 mmol), freshly distilled dichloromethane (40 mL), and chloroacetyl chloride (240 μL, 340.8 mg, 3.0 mmol). This mixture was stirred for 30 min at 0 °C and, subsequently, ferrocene (465 mg, 2.5 mmol) was added slowly to the reaction mixture as a solution in dichloromethane (20 mL). Stirring at 0 °C was continued for 2 h, after which the reaction was quenched by addition of water. After separation of the layers, the organic layer was washed with a saturated solution of $NaHCO_3$, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by chromatography over silica gel (eluent: *n*-pentane / diethyl ether 4 : 1) yielded three fractions: ferrocene (not isolated), **3.27** (124 mg (472 μmol) of orange crystals), and the corresponding diacylated product (45 mg (133 μmol) of a red oily substance). ^{1}H NMR ($CDCl_3$) δ 4.82 (d, *J* = 1.5 Hz, 2H), 4.58 (d, *J* = 1.8 Hz, 2H), 4.40 (d, *J* = 2.2 Hz, 2H), 4.23 (d, *J* = 2.2 Hz, 5H); ^{13}C NMR ($CDCl_3$) δ 195.3 (s), 73.1 (d), 70.2 (d), 69.5 (d), 46.0 (t); MS (EI+) m/z = 264 (M^+), 263 (M^+), 262 (M^+), 212, 185, 169, 158, 129, 121, 91, 78, 56.

*2-Chloro-1-ferrocenyl-1-ethanol (**3.28**)*



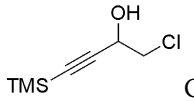
Obtained by reduction of ferrocenyl chloromethyl ketone (**3.27**) using sodium borohydride (0.5 equiv.) in methanol[37] as a red crystalline material in 94% yield. Subsequent purification was achieved by column chromatography over silica gel (eluent: *n*-pentane / diethyl ether 4 : 1). ^{1}H NMR ($CDCl_3$) δ 4.56 (ddd, *J* = 7.7, 4.0, 3.3 Hz, 1H), 4.28 (s, 1H), 4.22 – 4.19 (m, 8H), 3.69 (dd_{ABX}, *J* = 11.0, 4.0 Hz, $\Delta\nu_{AB}$ = 39.7 Hz, 1H), 3.59 (dd_{ABX}, *J* = 11.0, 7.7, $\Delta\nu_{AB}$ = 39.7 Hz, 1H), 2.42 (d, *J* = 3.3 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 89.1 (s), 70.0 (d), 68.7 (d), 68.3 (d), 67.2 (d), 65.8 (d), 49.6 (t); MS (EI+) m/z = 266 (M^+), 265 (M^+), 264 (M^+), 215, 187, 186, 163, 156, 138, 121, 108, 91, 65, 56; HRMS (EI+, for $C_{12}H_{13}{}^{56}Fe^{35}ClO$) calc. 264.0004, measured: 263.9999; chiral HPLC: Chiralcel AD, 40 °C, *n*-heptane / IPA 80 : 20, 1.0 mL/min, T_r = 7.6 min (*R*), T_r = 11.5 min (*S*).

*Trimethylsilanyl-propynal (**3.29**).*[38]



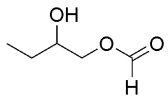
A flamedried 250 mL three-necked flask under an atmosphere of nitrogen was charged with freshly distilled diethyl ether (50 mL) and trimethylsilyl acetylene (3.56 mL, 2.46 g, 25 mmol), after which the temperature of the setup was lowered to –40 °C. Then, *n*-BuLi (2.5 M in hexane, 10 mL, 25 mmol) and DMF (2.33 mL, 2.20 g, 30 mmol) were added, respectively, and the mixture was stirred for 45 min while the temperature was allowed to increase to rt. The reaction mixture was then added to a vigorously stirring mixture of 10% aqueous NaH_2PO_4 (125 mL) and diethyl ether (125 mL). After separation, the aqueous layer was extracted once more with diethyl ether, the combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The resulting product (90% yield) was not purified further. The spectroscopic data of the obtained compound were in accordance with those given for **3.29** in the literature.[38]

*1-Chloro-4-trimethylsilanyl-but-3-yn-2-ol (**3.30**)*



Obtained by a literature procedure[15] as a colorless oil (1.54 g, 8.73 mmol, 87%) after column chromatography over silica gel (*n*-pentane – Et_2O 19 : 1). ^{1}H NMR ($CDCl_3$) δ 4.56 (ddd, J = 7.0, 5.9, 4.0 Hz, 1H), 3.70 (dd_{ABX}, J = 11.4, 4.0 Hz, $\Delta\nu_{AB}$ = 35.3 Hz, 1H), 3.61 (dd_{ABX}, J = 11.4, 7.0, $\Delta\nu_{AB}$ = 35.3 Hz, 1H), 2.37 (d, J = 5.9 Hz, 1H), 0.16 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 102.1 (s), 91.6 (s), 62.8 (d), 48.8 (t), –0.4 (q).

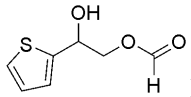
*2-Hydroxy-butyl formate (**3.31**).*[40,41]



A 100 mL two-necked flask was fitted with a distillation setup and charged with 1,2-butanediol (4.5 mL, 4.5 g, 50 mmol) and triethyl orthoformate (8.15 mL, 7.25 g, 48.9 mmol). This mixture was heated until no more ethanol evolved, then water (15 mL) was added and the mixture was stirred for 1.5 h at rt. Subsequently, the mixture was concentrated *in vacuo*, yielding 5.46 g (92%) of a 1.5 : 1 mixture of **3.31** and its regioisomer 1-hydroxy-butyl formate, respectively. After purification by column chromatography, this mixture was employed in kinetic resolution experiments. ^{1}H NMR ($CDCl_3$) δ 8.08 (s, 1H), 4.19 (dd_{ABX}, J = 11.4, 3.3 Hz, $\Delta\nu_{AB}$ = 51.4 Hz, 1H), 4.02

(dd_{ABX}, J = 11.4, 7.0, $\Delta\nu_{AB}$ = 51.4 Hz, 1H), 3.76 (ddd, J = 7.0, 5.9, 3.3 Hz, 1H), 2.40 (br s, 1H), 1.53 – 1.40 (m, 2H), 0.95 (t, J = 7.0 Hz, 3H).

*2-Hydroxy-2-thiophen-2-yl-ethyl formate (**3.32**)*



Prepared analogous to **3.31**, yielding a 5 : 1 mixture of **3.32** and its regioisomer 1-hydroxy-2-thiophen-2-yl-ethyl formate, respectively. This mixture was employed in kinetic resolution experiments after purification using column chromatography (*n*-pentane – Et_2O 2 : 1, 30%). ^{1}H NMR ($CDCl_3$) δ 8.11 (s, 1H), 7.30 (dd, J = 5.1, 1.1 Hz, 1H), 7.04 (ddd, J = 3.3, 1.1, 0.7 Hz, 1H), 6.99 (dd, J = 5.1, 3.3 Hz, 1H), 5.23 (dd, J = 7.7, 3.7 Hz, 1H), 4.42 (dd_{ABX}, J = 11.4, 3.7, $\Delta\nu_{AB}$ = 30.9 Hz, 1H), 4.35 (dd_{ABX}, J = 11.4, 7.7, $\Delta\nu_{AB}$ = 30.9 Hz, 1H), 2.58 (br s, 1H).

3.8.3 Production and purification of the enzyme[5a,47]

Solutions of purified HheC were prepared by C. Tarabiono. Halohydrin dehalogenase was expressed in *E. coli* MC1061. The *hheC* gene was amplified by PCR from pGEFHheC and cloned into pBAD/Myc-HisA between NcoI and PstI sites. Plasmid DNA was transformed by electroporation to *E. coli* cells, which were then plated on LB plates containing ampicillin and incubated overnight at 30 °C. A preculture was started by inoculating 100 mL of TB containing 50 μg/mL ampicillin with the transformants from a plate to a starting OD600 of 0.1. After overnight incubation at 30 °C, the preculture was diluted in 1 L of TB, containing 50 μg/mL ampicillin, 2.5 mM betaine, 0.5 M sorbitol and 0.02 % arabinose, and the culture was incubated for two days at 37 °C. The cells were centrifuged, washed, and resuspended in 50 mL of TEMG buffer (10 mM Tris-SO_4, 1 mM EDTA, 1 mM β-mercaptoethanol, and 10% glycerol, pH 7.5) containing a protease inhibitor cocktail (Complete Protease Inhibitor Cocktail Tablets, Roche). Cells were broken by sonication and the extract was centrifuged (50,000 rpm, 45 min, 4 °C). The supernatant was applied on a 50-mL Q-Sepharose anion exchange column and elution was carried out with a gradient of 0 to 0.45 M ammonium sulfate in TEMG. The collected fractions that displayed enzymatic activity were pooled and concentrated. The enzyme was stored at 4 °C for short-term storage or –20 °C for long-term storage.

3.8.4 General procedure for enzymatic kinetic resolution on analytical scale

To 20 mL of Tris-SO_4 buffer (100 mM, pH 8.1) at room temperature, 200 μL of a 1 M stock solution of substrate in DMF was added. Then, 20 μL of a solution of HheC in TEMG[c] of known activity[d] was added. Periodically, 1.0 mL aliquots were taken from the reaction mixture, which were extracted with 1.0 mL of toluene containing 5.0 mM of *n*-dodecane as an internal standard. The resulting organic solutions were then analyzed by chiral GC.

In case of substrate **3.5**, another internal standard was used (cinnamyl alcohol, present in the reaction mixture instead of the extraction solvent) as well as another extraction solvent, *n*-heptane. Reactions with this substrate were analyzed by chiral HPLC.

3.8.5 General procedure for enzymatic kinetic resolution on preparative scale

Typically, reactions were performed analogous to the procedure described for the kinetic resolutions on analytical scale, but on a scale of 1.0 – 2.0 mmol. This general procedure is for a reaction on 2.0 mmol scale. To 200 mL of Tris-sulfate buffer (100 mM, pH 8.1) at room temperature, 2.0 mL of a 1 M stock solution of substrate in DMF was added. Then, 50 μL of a solution of HheC in TEMG was added.[d] When the reaction had finished as determined by HPLC (**3.5**) or GC (the other substrates), the mixture was extracted with diethyl ether (or ethyl acetate if the goal was to isolate the formed diol as well), the combined organic layers dried on Na_2SO_4, filtered, and the solvents evaporated. The crude product(s) obtained were purified by column chromatography, using the conditions described for the racemic substrates. Details are given in Table 3.4.

Substrates **3.6** and **3.7** were resolved on a larger scale, in a two-phase system consisting of toluene in addition to aqueous Tris-sulfate buffer. Although some enzyme deactivation was observed under these conditions, it remained possible to perform these transformations using very low catalyst loadings: $1.5 \cdot 10^{-4}$ mol% and $3.0 \cdot 10^{-4}$ mol% for **3.6** and **3.7**, respectively.

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[c] The buffer solution consisted of 0.15 M $(NH_4)_2SO_4$ in TEMG (10 mM Tris-sulfate, pH 7.5, 3 mM EDTA, 0.1% 2-mercaptoethanol, 10% glycerol).

[d] The solution contained 3.0 – 6.0 mg/ml of active enzyme. Thus, the amount of HheC used was 60 – 120 μg for analytical scale resolutions and 150 – 300 μg for preparative scale resolutions. The actual amount of active HheC in each individual resolution was checked prior to the reaction by a spectrophotometric assay, according to a method described in: J. H. Lutje Spelberg, L. Tang, M. Van Gelder, R. M. Kellogg and D. B. Janssen, *Tetrahedron: Asymmetry* **2002**, *13*, 1083.

Table 3.4 Isolated yields and ee's for the kinetic resolution of **3.1** and **3.3** – **3.5**.

Substrate	Scale (mmol)	Enzyme (μg)	Reaction time (h)	Isol. y. chloroalcohol (mg, mmol, %)
(*S*)-**3.1**	1.0	200	5	47, 0.40, 40
(*S*)-**3.3**	2.0	250	16	100, 0.62, 31
(*S*)-**3.4**	1.5	300	66	70, 0.44, 29
(*S*)-**3.5**	2.0	740[b]	3	173, 0.95, 47

a) Ee's were >99% in each case; b) A large amount of enzyme was used to shorten the reaction time.

*Resolution of substrate **3.6***

To a mixture of 50 mL Tris-sulfate (2 M, pH 8.1) and 50 mL toluene was added a 1 : 1 w/w solution of racemic **3.6** (2.32 g, 16.1 mmol) in DMF. Next, 225 μg HheC was added. Since, after 8 h, the conversion as based on GC analysis turned out to proceed slower than expected, another 255 μg HheC was added, followed by another 176 μg after 32 h (total amount of enzyme: 656 μg). The reaction was stopped after 48 h. Flash chromatography (SiO_2, eluent *n*-pentane – Et_2O 4 : 1, $R_{f,chloroalc}$ = 0.33) yielded 989 mg (6.75 mmol, 42%) of (*S*)-**3.6** with an ee of 98.5%.

*Resolution of substrate **3.7***

A 1 : 1 v/v solution of racemic **3.7** (20.9 g; 129 mmol) in DMF was added to a mixture of 1 L Tris-sulfate (1 M, pH 8.1) and 100 mL toluene. Subsequently, a solution containing 2.68 mg of active HheC was added, followed by another 1.99 mg after 7 h, 0.88 mg after 24.5 h, 1.395 mg after 33.5 h, 1.395 mg after 54 h, 1.53 mg after 76 h, and 0.396 mg after 79.5 h (total amount of enzyme: 10.266 mg). After 4 d, the reaction mixture was extracted with toluene (3×). Subsequently, the combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*, yielding 9.8 g (60.3 mmol, 47%) of crude (*S*)-**3.7** with an ee of >99%. To extract the formed diol from the reaction mixture, the residual aqueous layer was evaporated and the resulting salt slurry extracted with dimethoxypropane and ethyl acetate, yielding 9.1 g (63.1 mmol, 49%) of nearly racemic 1-thiophen-2-yl-ethane-1,2-diol (**3.19**).

3.8.6 Determination of absolute configuration

The absolute configurations of the remaining enantiomers of the chloroalcohols were determined using several methods.

For (*S*)-(*E*)-1-chloro-4-phenyl-but-3-en-2-ol ((*S*)-**3.5**) a crystal structure (Cl used as heavy atom) was obtained (CCDC 605888).[27] Suitable crystals were obtained by slow diffusion of *n*-pentane into a concentrated solution of (*S*)-**3.5** in diethyl ether.

(*S*)-**3.6** and (*S*)-**3.7** could be correlated to known compounds by the sign of their optical rotation.[11,12]

3.6: $[\alpha]^{20}_D$ = +29.2 (c 0.452, $CHCl_3$), hence the configuration is *S* (lit. (*S*)-**3.6** $[\alpha]^{20}_D$ = +23.0 (c 0.52, $CHCl_3$)).[11]

3.7: Identified as (*S*)-**3.7** using the sign of rotation (+). (lit. (*S*)-**3.7** $[\alpha]^{20}_D$ = +28.5 (c 0.53, $CHCl_3$)).[11]

Compounds **3.1**, **3.3**, or **3.4** had not been described before in enantiomerically pure form. Therefore, they were hydrogenated to their saturated analogues **3.14** and **3.15**, for which optical rotations are known.[28] Since, in preliminary experiments, hydrogenation using Pd/C as a catalyst proved unsatisfactory, Wilkinson's catalyst ($Rh(PPh_3)_3Cl$) was employed.

The unsaturated chloroalcohol (1 mmol) was dissolved in 4 mL methanol together with 5 mol% of Wilkinson's catalyst, and this mixture was stirred until the catalyst had dissolved. After various vacuum / N_2 cycles the reaction mixture was put under an atmosphere of H_2 (25 bar) and was allowed to react overnight. Then it was filtered over a plug of silica, the solvent evaporated and the residue analyzed. After the product had been positively identified as the saturated chloroalcohol (NMR), the crude material was purified by flash chromatography and the optical rotation measured.

3.14 (from **3.1**): $[\alpha]^{20}_D$ = +1.3 (c 4.6, $CHCl_3$), hence the configuration is *S* (lit. (*S*)-**3.14** $[\alpha]^{20}_D$ = +1.1 (c 2.9, $CHCl_3$)).[28]

3.15 (from **3.3**): $[\alpha]^{20}_D$ = +1.1 (c 6.5, CHCl3), hence the configuration is *S* (lit. (*S*)-**3.15** $[\alpha]^{20}_D$ = +1.4 (c 3.1, $CHCl_3$)).[28]

3.15 (from **3.4**): Identified as (*S*)-**3.15** using the sign of rotation (+).[28]

3.9 Notes and references

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